

CLAIMS

1. A variant of a parent Fungamyl-like alpha-amylase, comprising an alteration at one or more regions selected from the group of:

- 5 Region 98-110,
 Region 150-160,
 Region 161-167,
 Region 280-288,
 Region 448-455,
10 Region 468-475.

wherein (a) the alteration(s) are independently

 (i) an insertion of an amino acid downstream of the amino acid which occupies the position,

 (ii) a deletion of the amino acid which occupies the position,

15 or

 (iii) a substitution of the amino acid which occupies the position with a different amino acid,

 (b) the variant has alpha-amylase activity and (c) each region or position corresponds to a region position of the amino acid
20 sequence of the parent Fungamyl-like alpha-amylase having the amino acid sequence of SEQ ID NO: 2.

2. The variant of claim 1, wherein the variant is one or more of the following substitution: Q153S.

25

3. The variant of claims 1, which variant has improved thermostability and/or increased stability at acidic pH.

4. A DNA construct comprising a DNA sequence encoding an alpha-
30 amylase variant of any of claims 1-3.

5. A recombinant expression vector which carries a DNA construct according to claim 4.

35 6. A cell which is transformed with a DNA construct according to claim 4 or a vector according to claim 5.

7. A cell according to claim 6, wherein the cell is a microorganism, such as a bacterium or a fungus.

5 8. The cell according to claim 7, which is a protease deficient strain of *Aspergillus*, in particular *A. oryzae*.

9. A composition for producing high maltose syrup comprising an Fungamyl-like alpha-amylase variant of claims 1-3.

10 10. The composition of claim 9, further comprising beta-amylase activity.

11. A dough improving composition, comprising an alpha-amylase
15 variant of any of claims 1-3.

12. A brewing composition comprising an alpha-amylase variant of any of claims 1-3.

20 13. The brewing composition of claim 12, further comprising one or more enzymes selected from the group of beta-amylase and isoamylase.

14. A composition for producing alcohol, comprising an alpha-
25 amylase variant of any of claims 1-3.

15. A process of liquefying starch, wherein an alpha-amylase variant of claims 1-3 is used for treating starch.

30 16. A process of producing high maltose syrups, wherein an alpha-amylase variant of claims 1-3 is used for liquefying starch.

17. A brewing process, wherein an alpha-amylase variant of claims 1-3 is added during fermentation of wort.

35 18. An alcohol production process, wherein an alpha-amylase

variant of claim 1-3 is used for liquefaction starch in a distillery mash.

19. A process, wherein a dough product comprising an alpha-amylase
5 variant of claims 1-3 is baked.

20. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for starch liquefaction.

10 21. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for producing alcohol.

22. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for brewing.

15 23. Use of an alpha-amylase variant of any of claims 1-3 or a composition of claim 9 for baking.

24. A method for generating an alpha-amylase variant of a parent
20 Fungamyl-like alpha-amylase, which variant has increased thermostability, in particular at acidic pH relative to the parent, the method comprising:

(a) subjecting a DNA sequence encoding the parent Fungamyl-like alpha-amylase to random mutagenesis,

25 (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

(c) screening for host cells expressing a mutated alpha-amylase which has improved thermostability at acidic pH relative to the parent Fungamyl-like alpha-amylase.

30 25. Process for producing a maltose syrup comprising the steps of:

1) liquefying starch in the presence of an alpha-amylase, followed by

35 2) dextrinization the presence of a fungal alpha-amylase variant of claim 1-3;

3) recovery of the syrup; and optional purification.

26. Process for producing syrup, in particular maltose syrup, comprising the steps of:

- 5 1) liquefying starch at a temperature of 140-160°C at a pH of 4-6, followed by
2) dextrinization at a temperature in the range from 60-95°C at a pH 4-6 in the presence of a fungal alpha-amylase variant of claims 1-3; and
10 3) recovery of the syrup; and optional purification.

27. The process of claim 26, wherein the liquefying starch is treated at a temperature of 65-85°C, in particular 70-80°C.

- 15 28. The process of claim 27, wherein an effective amount of glucoamylase is added in step 2).

29. Process for producing maltose syrup, comprising the steps of:

- 20 1) liquefying starch at a temperature of 95-110°C at a pH of 4-6 in the presence of a *Bacillus* alpha-amylase, followed by
2) dextrinization at a temperature in the range from 60-95°C at a pH 4-6 in the presence of a fungal alpha-amylase variant of claims 1-3; and
3) recovery of the syrup; and optional purification.

25

30. An immobilized variant of claims 1-3.